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THE ARCHEGONIUM AND SPOROPHYTE OF TREUBIA INSIGNIS GOEBEL

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One of the largest and most interesting liverworts is *Treubia insignis* discovered by Goebel in western Java, and named by him for the late distinguished director of the famous botanical gardens at Buitenzorg in Java, Dr. Melchior Treub.

The plant was collected near Tjibodas on Mt. Gedeh, a volcano in western Java, and it has since been found repeatedly by various botanists in this neighborhood. Schiffner[1] gives this as the only known habitat, but later collectors have found the plant (or a closely related species) in several widely separated regions. Goebel himself collected a Treubia in New Zealand, and it has been reported from Tasmania, Tahiti, Samoa and Patagonia.

"Stephani"[2] recognizes two species, *T. insignis*, from Java and Tahiti, and *T. bracteata* from Samoa. Sterile material only has been found in this latter species. *T. bracteata* has recently been reported from Tasmania,[3] and it is not unlikely that the New Zealand species is the same.

In May, 1913, the writer collected a single specimen of Treubia on Mt. Banajao in Luzon, Philippine Islands. The specimen was sterile, but except for its somewhat smaller size, it seemed to be identical with material collected in Java.

During a visit to Java in 1906 the writer secured a large amount of material near Tjibodas, where the plant was found growing in some places in great profusion on the ground and on rotten logs. Only a few plants with sporogonia could be found, but a number of plants

[1] Die Hepaticae der Flora von Buitenzorg. Leiden. 1900.

[2] Stephani, F. Species Hepaticarum. Mém. Herb. Boiss., **16**. 1900.

[3] Rodway, L. Notes on *Treubia insignis* Goebel. Papers Proc. Roy. Soc. Tasmania.

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bearing archegonia were secured, and many plants with the characteristic gemmae. No antheridial plants were found in the material collected, and it is probable that the rarity of male plants accounts for the small number of fertilized female plants.

Treubia not only is one of the largest liverworts, but it shows a number of interesting structural features which have been pretty thoroughly investigated by Goebel[4] and more recently by Grün.[5]

It has a thick fleshy midrib or axis, and develops two rows of very large and distinct leaves of the "succubous" type, *i. e.*, the hinder margin of a leaf overlaps the forward margin of the next older leaf (fig. 1, *A*). At the base of each leaf, at its junction with the axis, a

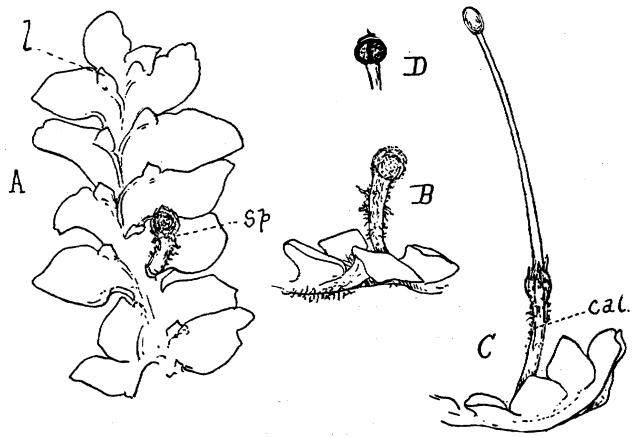

FIG. 1. *A*. Plant of *Treubia insignis* Goebel, × 1½. *sp*, sporophyte, still enclosed in the calyptra; *l*, dorsal scale. *B*. The sporophyte seen from the side. *C*. Plant with mature sporophyte, × 1½; *cal*, calyptra. *D*. An open capsule, × 3.

conspicuous appendage or scale (1) is formed upon the dorsal side, and this scale in the fertile plants covers the groups of archegonia and antheridia. These scales are connected by ridges which form a zigzag line along the center of the axis. In many plants there are produced groups of gemmae in the same position as the sexual organs. No amphigastria or ventral appendages can be seen.

[4] Goebel, K. Morphologische und biologische Studien IV. Ann. Jard. Bot. Buitenzorg 9. 1891.

[5] Grün, C. Monographische Studien an *Treubia insignis* Goebel. Flora 106. 1914.

The shoot branches monopodially, but neither Goebel nor Grün determined exactly the origin of the lateral branches, nor their relation, if any, to the leaves. The plant may reach a length of 16 cm. with an extreme breadth of 2.5 cm.

Although the general aspect of the plant is that of a very large acrogynous leafy liverwort, in the position of the archegonia and sporophyte, it is distinctly anacrogynous, *i. e.*, the apical cell of the shoot is not transformed into an archegonium. Unlike most of the Anacrogynae, *e. g.*, Pellia, Mörkia, Pallavicinia, etc., the archegonia do not arise in the median plane of the shoot but are formed in lateral groups subtended by the scales at the base of the leaves. Goebel compares their position to that in Fossombronia, where the archegonia are also lateral in position; but in the latter the archegonia are formed singly and not in groups, and instead of being protected by a distinct scale are covered only by the inrolled margin of the young leaf.

Goebel showed that the growth of the shoot is due to a three-sided pyramidal apical cell, very much like that of the typical Acrogynae, and his statement has been verified by Grün. As in most leafy liverworts the ventral face of the apical cell is smaller than the two dorsal lateral faces. From the latter, segments are cut off, each of which gives rise to a leaf, but no trace of leaves (amphigastria) are produced from the ventral segments.

The leaves are very large, and, except for the extreme margin, are composed of several layers of cells. From the ventral side of the leaf is developed a wing-like outgrowth which extends for a short distance along the ventral surface of the axis. On this wing are developed many mucilage-secreting papillae which exude great quantities of a colorless slime. Goebel suggests that the abundance of these secreting organs on the leaves accounts for the absence of the secretory hairs or scales that are so commonly found on the ventral surface of the apical region in most thallose liverworts. These secretory papillae may be single cells, or they may be stalked organs. The mucilaginous secretion fills a shallow furrow which occupies the ventral side of the midrib, and within this furrow are numerous short rhizoids. Goebel found in some of the cells of the thallus oil-bodies much like those occurring in the Marchantiales. Similar, but smaller oil-bodies occur also in many other liverworts.

There is always present an endophytic fungus which is very abundant in the ventral region of the shoot, and mainly confined to a

definite zone just above the shallow furrow already referred to. The writer made no special study of this mycorrhiza which is evidently very similar to that found in a number of other liverworts, as well as in the gametophytes of a good many ferns, notably the Marattiaceae.

Goebel discusses at some length the nature of the dorsal scales which protect the reproductive organs but does not come to a definite conclusion. He thinks they may be considered either as independent structures, or as part of the leaf. According to Goebel's account, which has been confirmed by Grün, the young segment of the apical cell, from which this leaf arises, shows, when seen from the surface, three cells, of which two give rise to the leaf, and one—that nearest the midrib—to this scale. The relation of the leaf and scale is therefore the same as that of the two lobes found in the leaves of so many acrogynous liverworts, and it seems to the writer that this is probably the simplest interpretation of the case in Treubia.

Treubia is in several respects much like Fossombronia. This is true of the origin of the leaves, and in the position of the archegonia. Fossombronia, like Treubia, usually is infested by a mycorrhizal fungus—at least this is true for *F. longiseta*,[6] which also shows small oil-bodies in some of the leaf-cells. These, however, are much less conspicuous than the large oil-bodies of Treubia. Fossombronia differs from Treubia in the form of the apical cell,[7] which is of the two-sided type found in moss Anacrogynae. One of Humphrey's figures of *F. longiseta*,[8] suggests the possible occurrence of a three-sided apical cell in this species.

Another liverwort which has the same type of apical cell as Treubia is Noteroclada (Androcryphia), and Schiffner,[9] who has studied this rare liverwort, concludes that it is nearly related to Treubia with which it agrees not only in the form of the apical cell, but also in its leaf structure. Schiffner thinks that Noteroclada is also related to Fossombronia, with which it is connected by Petalophyllum.

The Archegonium

Goebel made no special study of the archegonium, but Grün has given a pretty satisfactory account of its most important features,

[6] Humphrey, H. B. The Development of *Fossombronia longiseta*. Annals of Botany, 20. 1906.

[7] Leitgeb, H. Untersuchungen über die Lebermcose, Heft III. Jena. 1877.

[8] Humphrey. L. c. Text-fig. 4, *H.*

[9] Schiffner. Zur Morphologie von Noteroclada. Österr. Bot. Zeitschr. **61.** 1911.

which have been confirmed for the most part by the writer's own observations.

The archegonia occur in groups, sometimes as many as a dozen together. As we have already seen, they are laterally placed, and each group is in the axil of one of the dorsal scales, which completely covers it.

The youngest archegonia (fig. 2, *A*) are hemispherical cells. The first division may be a horizontal one, cutting off a short stalk-cell—or it may be strongly oblique. In the latter case the first wall is quickly followed by two similar ones, which intersect so that a central (terminal) cell is formed which appears triangular in longitudinal

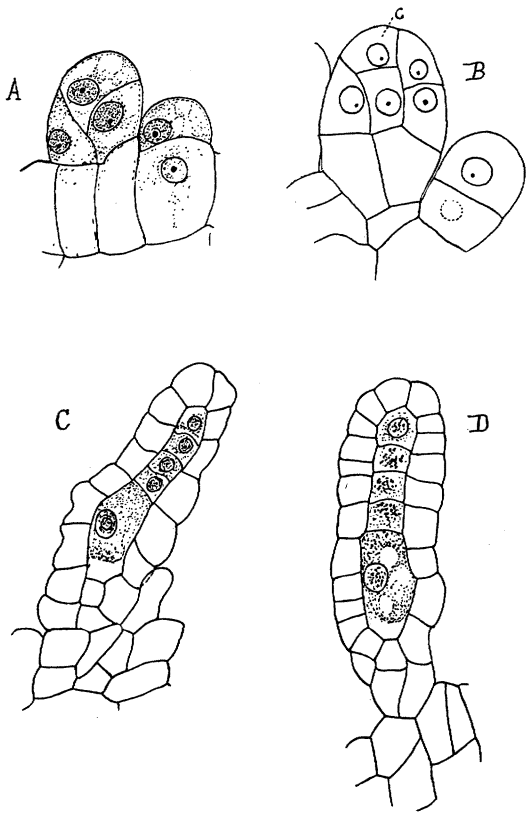

FIG. 2. *A*. Two very young archegonia, × 550. *B*. A somewhat older stage, showing the cap-cell, *c*, × 550. *C*, *D*. Older stages, showing four neck canal-cells, × 335.

section. Where a stalk cell is first cut off, this is followed by three nearly vertical intersecting walls in the terminal cell. The central cell in this case is truncate below (fig. 2, *B*) instead of pointed. In either case the next division is usually at least a transverse wall in the central cell, cutting off a cap-cell (fig. 2, *B*, *c*), which finally is divided into four by intersecting vertical walls.

The subsequent divisions in the archegonium follow the usual course; *i. e.*, a series of transverse divisions, in all but the cap-cell,

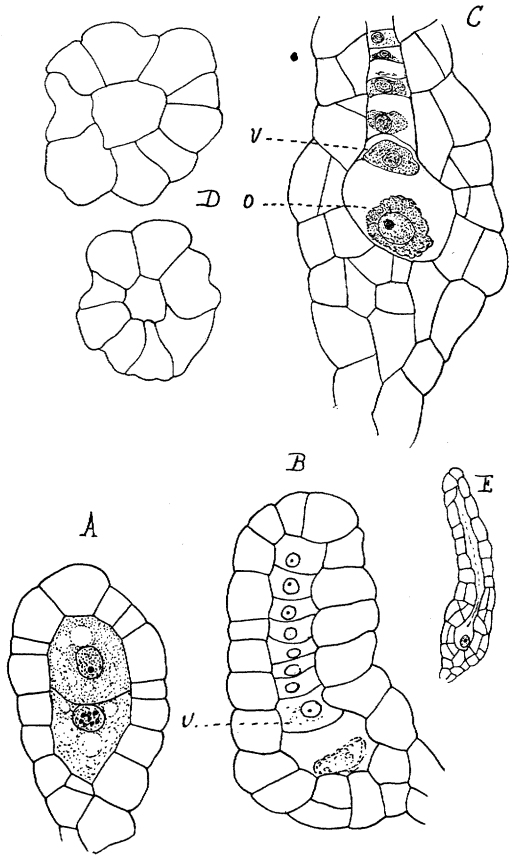

FIG. 3. *A*. Young archegonium, showing the separation of the central cell and the primary neck-canal cell, × 550. *B*. An older archegonium with seven neck-canal cells, and ventral canal-cell, *v*. *C*. The venter of a nearly mature archegonium. *o*, egg; *v*, ventral canal-cell, × 550. *D*. Two cross sections of the archegonium. Neck, × 550. *E*. Adult archegonium, × about 100.

separates the lower or ventral region from the neck; and in the three primary peripheral cells of the neck, a longitudinal division inaugurates six rows of neck-cells. In the older archegonium of Treubia, however, other longitudinal walls may appear, so that a cross-section of the neck, especially in its lower part, shows sometimes as many as nine peripheral cells, while most of the Jungermanniales have but five. As a result of this increased number there is not a clearly marked line between the venter and the base of the neck (fig. 3, *C*, *D*).

Figure 2, *C*, *D* show longitudinal sections of two young archegonia in which the central cell of the venter is still undivided, and the primary neck canal-cell has divided twice. As in other liverworts, there is later cut off from the central cell, the ventral canal-cell (fig. 3, *C*, *v*), and there is a further division of the neck canal-cells. None of the specimens examined showed more than eight neck canal-cells, but Grün gives a figure showing sixteen, which he says is the normal number for the fully developed archegonium.

The cells forming the wall of the venter undergo periclinal divisions, so that at maturity the egg is surrounded by a double layer of cells.

The number of archegonia in a group, in the specimens examined by the writer, was about a dozen. Mingled with the archegonia are numerous paraphyses ("Paraphylls"), which may be either simple cell-rows, or more or less expanded and branched scales. The marginal cells of these scales are often secretory organs, exuding a mucilaginous matter like that developed from the mucilage papillae on the lower side of the leaves.

The Embryo

Grün has described somewhat at length the structure of the older sporophyte, but he was unable to get the earliest stages. The account here given is far from complete, owing to the limited amount of material that was available; but it is hoped that it will be sufficient to make clear the most important points in the early history of the sporophyte.

The earliest divisions were not seen, but it is pretty certain that they are transverse as in all other Jungermanniales that have been investigated. It is also reasonably certain that the lowermost segment (or segments?) are devoted to the formation of the conspicuous haustorium which is a marked feature of the young embryo. All of

the structures of the older sporophyte, foot, seta and capsule, are presumably developed from the terminal cells of the young embryo, as they are in other similar forms, *e. g.*, Podomitrium, Pallavicinia, etc.

Figure 4, *A*, shows a young sporophyte in which the basal region constitutes a haustorium made up of large cells. The embryo at this

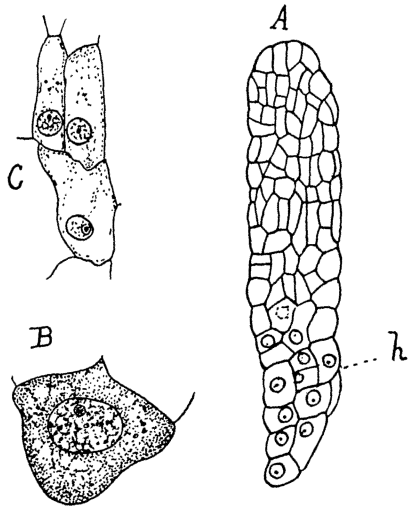

FIG. 4. *A*. Young sporophyte showing the large haustorium, *h*, × 84. *B*. A single cell of the haustorium, showing the large nucleus, × 375. *C*. Cells from the upper part of the sporophyte, × 375.

stage closely resembles that of certain species of Pallavicinia,[10] but the haustorial cells are relatively smaller and more numerous. The cells of the haustorium have very much larger nuclei than those of the upper part of the embryo (fig. 4, *B*, *C*). In the later stages of development these haustorial cells become very much compressed by the rapid growth of the foot of the young sporophyte, which evidently replaces them as an organ of absorption. A similar condition was noted by the writer in Podomitrium.[11]

As the sporophyte grows the lower portion enlarges slightly to form the rather indefinite foot (fig. 5, *C*, *f*) while the terminal region,

[10] Campbell, D. H. and Williams, F. A Morphological Study of Some Members of the Genus Pallavicinia. Stanford University. 1914.

[11] Campbell, D. H. The Morphology and Systematic Position of Podomitrium. Amer. Journ. Bot. **2**, 199. 1915.

which is only slightly broader than the intermediate portion (seta), begins to show the first evidences of a differentiation of sporogenous tissue.

In the development of the primary sporogenous tissue Treubia resembles most nearly, of the forms that have been investigated, Podomitrium. As in the latter the young sporogenous tissue is very vaguely defined, and it is quite impossible to determine exactly its extent (fig. 5, *A*). In this respect Treubia differs from Pallavicinia,

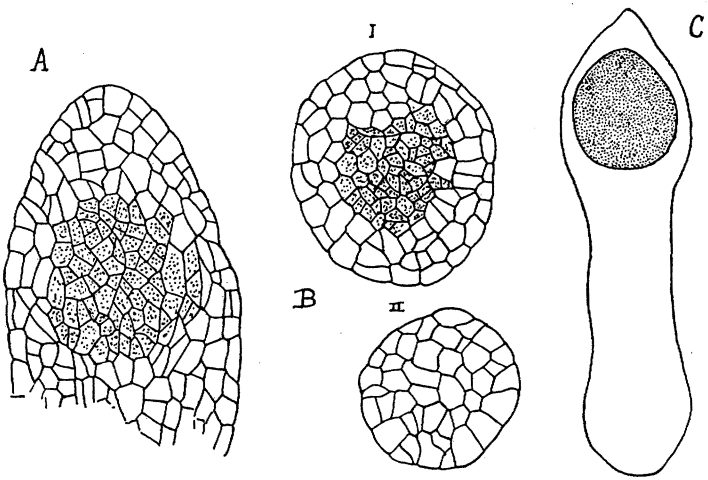

FIG. 5. *A*. Upper part of young sporophyte, × 84; the sporogenous tissue is shaded. *B*. Two cross-sections of a sporophyte of about the same age as that shown in *A*. *C*. Longitudinal section of an older sporophyte, × 27.

Aneura or Fossombronia, where the limits of the young archesporium are much more definite, this being especially marked in Aneura and Fossombronia, where the archesporium is recognizable at a very early stage of development.

The sporogenous region is bounded by several layers of sterile tissue, which form the wall of the capsule. This is about three cells thick at the sides, but at the apex of the capsule there may be as many as eight, and a conspicuous beak is produced as in Pallavicinia and Podomitrium, and to a lesser degree in Calycularia (fig. 6, *A*). In this respect Treubia also differs from Fossombronia and Aneura where the apical part of the capsule wall (aside from the elaterophore) is of the same thickness as the lateral wall.

The sporogenous region becomes more clearly defined as cell-division proceeds, and there is soon to be made out a distinction between spore mother-cells and elaters. No definite relation could be made out between these. The elaters occur either singly or in small groups irregularly distributed among the very much more numerous young spore mother cells (fig. 6, *B*). A few elaters could

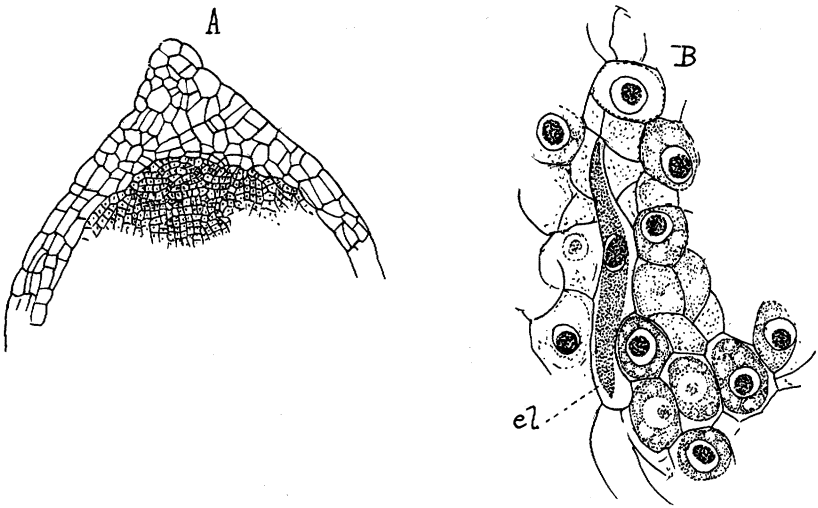

FIG. 6. *A*. Upper part of a young sporophyte showing the beak-like prominence at the apex, × 60. *B*. The sporogenous tissue from an older sporophyte showing a young elater (*el*) and the young spore mother-cells, × 400.

often be found radiating from the base of the capsule, but there was nothing which could be described as a definite elaterophore. The elaters are less numerous than is usual among liverworts, but they finally attain a length which probably exceeds that of any other known form.

Grün[12] states that the rounding off of the spore mother-cells and the spaces between them which are seen in the later stages are partly due to the disintegration of some of the sporogenous cells; a careful examination of the writer's preparations of these stages, which were well fixed and stained, showed no evidence of the breaking down of any of the cells, and it seems practically certain that the separation of the elaters and the rounding off of the spore mother-

[12] L. c., p. 372.

cells can be perfectly explained as the result of a partial dissolution of the cell-walls, together with the rapid enlargement of the capsule in the later stages of development, which is not accompanied by a corresponding increase in the size of the spore mother-cells.

Lack of material made it impossible to study the spore division, as no stages were found between that shown in figure 6, *B*, which shows the young spore mother-cells before the final divisions had begun, and nearly ripe spores. The material was fixed in acetic alcohol, so that the finer details of the nuclear structures were not very satisfactorily shown. The nuclear contents were often contracted, especially in the spore mother-cells; but whether this was a normal synapsis, or, what is more likely, the result of imperfect fixation, was not determined.

Grün succeeded in finding the dividing spore mother-cells, although not a sufficient number of stages to make out all the details. He found no indication of centrosomes such as Farmer[13] describes for *Pallavicinia decipiens*, and some indications of which were found by the writer in *Calycularia radiculosa*. Grün found sixteen chromosomes in the dividing cells of the sporogenous tissue before the final divisions of the spore mother-cells, but it is not clear just where the reduction division occurs. To judge from his account and figures, it seems that in *Treubia insignis*, as in *Pallavicinia decipiens* and *Calycularia radiculosa*, there is a "quadripolar" spindle, and not two successive bi-polar spindles such as usually are found in spore division.

The calyptra (fig. 1, *C*, *cal.*) enclosing the developing sporophyte is very large in Treubia, where it may reach a length of nearly 1.5 cm. and in also very massive. The surface develops scale-like outgrowths which give it a shaggy appearance, and among the scales may be seen the remains of the unfertilized archegonia. The fully developed sporophyte has a seta about 35 mm. in length and the ovoid capsule is about 2.5 mm. in length (fig. 1, *C*). The capsule dehisces by four somewhat irregular valves (fig. 1, *D*).

Andreas[14] has given a fairly complete account of the structure of the wall of the mature capsule, and Grün has supplemented this by a careful study of the development of the wall in the later stages.

[13] Farmer, J. B. On *Pallavicinia decipiens*. Annals of Botany, 8. 1904.

[14] Andreas, J. Über den Bau der Wand und die Öffnungsweise des Lebermoossporogons. Flora 86. 1899.

Except for the apex, which as we have seen has a conspicuous beak formed of several (8–10) layers of cells, the capsule wall is usually composed of three layers. In the earlier stages these layers are composed of uniform cells, but as development proceeds the two inner layers undergo more or less numerous divisions while the cells of the superficial layer remain undivided and increase much in size as the capsule enlarges. On the walls of the two inner cell-layers characteristic thickenings are formed while the walls of the superficial cells undergo little change. The thickenings on the walls of the inner cells are of various kinds—ridges, complete rings, half-rings, and spirals. Grün states that these cells contain chlorophyll and starch granules. The ripe capsule is ovoid in outline, and not globular, as Andreas states. It opens by four short and somewhat irregular valves. Grün examined the ripe spores and elaters. The former have reticulate thickenings upon the surface, and resemble the spores of certain species of Pallavicinia. They measure from 20 to 25 μ in diameter. The elaters reach the extraordinary length of 1,250 μ.

Conclusion

Most writers agree that Treubia has much in common with the acrogynous leafy liverworts and in a sense connects them with the typical anacrogynous forms. Among the latter, the genera Fossombronia, Ptalophyllum and Noteroclada are most nearly related to Treubia. Cavers[15] in his recent summary of the Hepaticae considers these to have been derived from Pellia-like ancestors, but he looks upon Fossombronia as most nearly related to the Acrogynae.

It seems more likely that Treubia is nearer to the Acrogynae than is Fossombronia. This is true of the character of the leaves, the apical cell, and the groups of archegonia. It is by no means impossible that the dorsal scales may be the homologue of the dorsal lobe of such leafy liverworts as show a bilobed leaf, *e. g.*, Madotheca, Frullania, etc. Schiffner,[16] who has studied Noteroclada concludes that it is closely related to Treubia and must be considered as the end of a series of which Fossombronia is a lower member.

Fossombronia differs a good deal from the Pellia type, and is in

[15] Cavers, F. The Inter-relationships of the Bryophyta. New Phytologist Reprint, No. 4. Cambridge, 1911.

[16] L. c.

some respects much more like Geothallus, which in turn is unmistakably closely related to Sphaerocarpus. It is possible that the Fossombronia line (including Petalophyllum, Noteroclada and Treubia) is a direct development of the Sphaerocarpus type and is not closely related to the Pellia line (Codoniaceae). Moreover it is not unlikely that from the Fossombronia line, the Acrogynae (or part of them) have originated. Should this hypothesis be correct, it would necessitate the removal of the series of forms—Fossombronia, Treubia—from the Codoniaceae and their association with the Sphaerocarpales.